RESEARCH PAPER

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Impact of vitamin D administration on immunogenicity of trivalent inactivated influenza vaccine in previously unvaccinated children

Nicola Principi,[1] Paola Marchisio,[1] Leonardo Terranova,[1] Alberto Zampiero,[1] Elena Baggi,[1] Cristina Daleno,[1] Silvia Tirelli,[2] Claudio Pelucchi[3] and Susanna Esposito[1,*]

[1]Pediatric Clinic 1; Department of Pathophysiology and Transplantation; Università degli Studi di Milano; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; Milan, Italy; [2]Laboratory Medicine Unit; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; Milan, Italy; [3]Department of Epidemiology; Istituto di Ricerche Farmacologiche Mario Negri; Milan, Italy

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As vitamin D (VD) has a significant regulatory effect on innate and adaptive immunity, the aim of this prospective, randomized, single-blinded, placebo-controlled study was to measure the impact of VD administration on the immune response to trivalent influenza vaccination (TIV). A total of 116 children (61 males, 52.6%; mean age 3.0 ± 1.0 y) with a history of recurrent acute otitis media (AOM), who had not been previously vaccinated against influenza, were randomized to receive daily VD 1,000 IU or placebo by mouth for four months. All of them received two doses of TIV (Fluarix, GlaxoSmithKline Biologicals) one month apart, with the first dose administered when VD supplementation was started. There was no difference in seroconversion or seroprotection rates, or antibody titers, in relation to any of the three influenza vaccine antigens between the VD and placebo groups, independently of baseline and post-treatment VD levels. The safety profile was also similar in the two groups. These data indicate that the daily administration of VD 1,000 IU for four months from the time of the injection of the first dose of TIV does not significantly modify the antibody response evoked by influenza vaccine.

Introduction

As a number of studies have clearly demonstrated that the active form of vitamin D (VD), calcitriol (1,25-dihydroxyVD), has a significant regulatory effect on normal innate and adaptive immunity,[1] it was believed that VD status could condition the immune response to vaccines. This hypothesis seemed to be confirmed by the findings of experimental animal studies indicating that the addition of VD or calcitriol to a variety of vaccine preparations could increase induced immunity to herpes simplex virus, diphtheria toxoid, tetanus toxoid, hepatitis B surface antigen, poliovirus, influenza viruses and HIV gp160.[2-7]

However, the collected human data are controversial. Zitt et al. found that VD deficiency was associated with a poor response to active hepatitis B immunisation in adult patients with chronic kidney disease,[8] whereas Heine et al. found that the induction of specific tetanus toxoid immunoglobulin after booster immunisation was significantly greater in adults receiving VD supplementation than in untreated controls.[9] The results of studies of influenza vaccine are equally conflicting. Chadha et al. found that a replete VD status was associated with a more frequent response to trivalent inactivated influenza vaccine (TIV) in prostate cancer patients,[10] whereas the findings of Kriesel and Spruance[11] and Cooper et al. were the opposite:[12] the former co-administered calcitriol and TIV to adult volunteers and did not find any significant effect of VD supplementation on humoral immunity,[11] and the latter found that VD did not have any positive effect on specific antibody production after the administration of TIV to HIV-infected adults.[12]

Discovering whether VD supplementation can enhance the protection evoked by TIV may be important because of the relatively poor immune response of some subjects (particularly younger children and elderly adults) and the fact that attempts to increase immunogenicity in different ways have frequently led to debatable results.[13]

We have recently evaluated the impact of VD administration on the incidence of new episodes of acute otitis media (AOM) in children with a history of recurrent AOM and, as all of the children enrolled in this study were administered a TIV, we took the opportunity to measure the impact of VD on their immune responses to the vaccination.

*Correspondence to: Susanna Esposito; Email: susanna.esposito@unimi.it
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Table 1. Immune responses to a trivalent influenza vaccine in a previously unvaccinated pediatric population by vitamin D levels before vitamin D treatment

Immune response	Vitamin D < 20 ng/mL (n = 23)	Vitamin D 20–29.9 ng/mL (n = 60)	Vitamin D ≥ 30 ng/mL (n = 33)	P value
A/H1N1v				
Seroconversion, No. (%)	12 (52.2)	28 (46.7)	11 (33.3)	0.31
Seroprotection (HI ≥ 40 at T1), No. (%)	18 (78.3)	48 (80.0)	27 (81.8)	0.95
Seroprotection (HI ≥ 110 at T1), No. (%)	11 (47.8)	31 (51.7)	19 (57.6)	0.76
Median GMT at T1 (range)	80 (20–2,560)	160 (5–2,560)	160 (5–2,560)	0.76
A/H3N2				
Seroconversion, No. (%)	11 (47.8)	35 (58.3)	16 (48.5)	0.55
Seroprotection (HI ≥ 40 at T1), No. (%)	20 (87.0)	50 (83.3)	27 (81.8)	0.87
Seroprotection (HI ≥ 110 at T1), No. (%)	16 (69.6)	36 (60.0)	19 (57.6)	0.64
Median GMT at T1 (range)	640 (10–5,120)	160 (5–2,560)	160 (5–2,560)	0.33
B				
Seroconversion, No. (%)	6 (26.1)	17 (28.3)	10 (30.3)	0.94
Seroprotection (HI ≥ 40 at T1), No. (%)	7 (30.4)	21 (35.0)	11 (33.3)	0.92
Seroprotection (HI ≥ 110 at T1), No. (%)	2 (8.7)	4 (6.7)	4 (12.1)	0.67
Median GMT at T1 (range)	10 (5–640)	10 (5–160)	10 (5–320)	0.54

P value between groups calculated using the x^2 or Kruskall-Wallis test as appropriate. GMT, geometric mean titer; HI, hemagglutination-inhibiting antibodies. T1, end of the treatment period, three months after the last TIV administration.

Results

The study involved 116 children (61 males, 52.6%; mean age 3.0 ± 1.0 y), none of whom had been previously vaccinated against influenza: 59 (50.9%; mean age 3.3 ± 1.1 y) were administered VD and 57 (49.1%; mean age 2.9 ± 0.9 y) received placebo. The mean baseline VD concentrations were similar in the two groups: 27.4 ± 26.2 vs. 25.8 ± 25.9 ng/mL (p = 0.62).

Table 1 shows the immune responses to TIV by VD levels before treatment. The responses of the children with VD deficiency were similar to those of the children with normal values.

Table 2 shows the immune responses to the three antigens included in the TIV in the two treatment groups. Despite children receiving VD had at the end of treatment a significantly higher mean VD level than children given placebo (36.8 ± 8.5 ng/mL vs. 18.7 ± 7.0 ng/mL, p < 0.001), there was no between-group difference in seroconversion or seroprotection rates in relation to any of the influenza antigens, regardless of the cut-off value used to define seroprotection.

Table 3 shows the immune response to TIV by VD levels after treatment. Once again, there was no between-group difference in seroconversion or seroprotection rates in relation to any of the influenza antigens, regardless of the cut-off value used to define seroprotection. The immune response to all three influenza viruses was independent of the final VD level and, consequently, independent of VD supplementation. Moreover, no difference in immune response was found between children with persistently low (< 20 ng/mL) and those with persistently high VD levels (VD > 30 ng/mL). TIV was well tolerated in the two groups and there was no between-group difference in the mild and transient local and systemic adverse events. Local reactions were reported in 7 (11.9%) children treated with VD and in 6 (10.5%) of those who received placebo: in all the cases they were recorded in the first two days after vaccine administration, symptoms lasted no more than two days and swelling/induration was the most common local event in both the groups (10.2% in VD group vs. 7.0% in placebo group). Systemic reactions were reported in 10 (16.9%) children treated with VD and in 9 (15.8%) of those who received placebo: they were recorded in the first five days after vaccine administration, symptoms lasted no more than three days, irritability was the most common systemic event in both the groups (11.9% in VD group vs. 10.5% in placebo group) and fever ≥ 38°C was registered in few cases (5.1% in VD group vs. 5.2% in placebo group). No serious adverse event was reported.

Discussion

Influenza is common in pediatrics, and it causes a considerable number of clinical, social and economic problems, not least because children are the main cause of the spread of the infection.[14] Patients with chronic and severe underlying diseases are at the highest risk of influenza-related complications, but otherwise healthy subjects can also develop severe influenza, particularly if they are less than five years of age.[15,16] The potential severity of the disease and the role played by children in spreading the infection explain why influenza vaccination is strongly recommended throughout the world for children with chronic and severe underlying diseases and, in an increasing number of countries, for at least the youngest healthy subjects.[17,18] Unfortunately, the protection induced by TIV in younger children is not optimal because of the immaturity of the immune system in the first years of life. The methods used to increase immunogenicity of TIV in older adults are not licensed for use in children, with the exception of

Table 2. Immune responses to a trivalent influenza vaccine in a previously unvaccinated pediatric population receiving vitamin D treatment or not

Immune response	With vitamin D (n = 59)	With Placebo (n = 57)	P value
A/H1N1v			
Seroconversion, No. (%)	24 (40.7)	27 (47.4)	0.47
Seroprotection (HI ≥ 40 at T1), No. (%)	48 (81.4)	45 (79.0)	0.75
Seroprotection (HI ≥ 110 at T1), No. (%)	31 (52.5)	30 (52.6)	0.99
Median GMT at T1 (range)	160 (5–2,560)	320 (5–2,560)	0.79
A/H3N2			
Seroconversion, No. (%)	30 (50.9)	32 (56.1)	0.57
Seroprotection (HI ≥ 40 at T1), No. (%)	49 (83.1)	48 (84.2)	0.87
Seroprotection (HI ≥ 110 at T1), No. (%)	35 (59.3)	36 (63.2)	0.67
Median GMT at T1 (range)	160 (5–5,120)	160 (5–2,560)	0.72
B			
Seroconversion, No. (%)	16 (27.1)	17 (29.8)	0.75
Seroprotection (HI ≥ 40 at T1), No. (%)	19 (32.2)	20 (35.1)	0.74
Seroprotection (HI ≥ 110 at T1), No. (%)	2 (3.4)	8 (14.0)	0.051
Median GMT at T1 (range)	10 (5–160)	10 (5–640)	0.54

P value between groups calculated using the x2 or Wilcoxon rank-sum test as appropriate. GMT, geometric mean titer; HI, hemagglutination-inhibiting antibodies. T1, end of the treatment period, three months after the last TIV administration.

virosome adjuvanted TIV in some countries.[13] Consequently, the possible role of VD as a modulator of immune responses to TIV is particularly important in pediatrics.

This is the first study to evaluate this aspect. Because it was a by-product of another study, the immune responses to TIV were assessed later than in studies specifically performed for this purpose (i.e., after three instead of one month), which could have led to an underestimate of seroconversion and seroprotection rates. However, as the aim of the study was to compare the effect of VD supplementation on TIV-induced antibody production, we believe that the time of evaluation does not affect the conclusions that can be drawn from the data even though they refer more to the persistence of protection rather than immediate antibody production.

Unfortunately, the findings are highly disappointing because they seem to indicate that the daily administration of VD 1,000 IU for four months after the injection of the first dose of TIV does not significantly change vaccine-induced antibody responses, regardless of pre-existing immunity against influenza or the cut-off level used to define seroprotection. Paradoxically, at the end of treatment period the number of children achieving the higher cut-off level for seroprotection against influenza B virus was greater among those who received placebo than among subjects treated with VD with a difference having a p value in proximity to statistical significance.

The lack of any positive effect of the simultaneous administration of VD and TIV to subjects with different baseline influenza antibody titers has been previously reported by Kriesel and Spruance, who studied healthy adults administered calcitriol 1 μg i.m. or a saline placebo at a site adjacent to the vaccine injection.[11] They suggested that their negative findings could be attributed to the fact that the active form of VD was given simultaneously with TIV, and that this could have masked its potential immunomodulatory effect. However, as all of the children in our study were unprimed, they were given two vaccine doses separated by an interval of four weeks and continued to receive VD during this period. Nevertheless, despite this and the increase in serum VD levels, their immune response to TIV was quite similar to that of the children in the placebo group whose serum VD levels became lower on average. However, it cannot be excluded that the persistence of normal levels in the subjects receiving VD may have been too short to reveal its immunomodulatory effect.

A second possible reason for the negative results of VD administration is the dose, although the daily dose of 1,000 IU was high enough to restore normal serum VD levels in the children who were VD deficient at baseline, which suggests that the dose was adequate. However, despite this, there was no significant change in TIV immunogenicity.

A number of the children had lower than normal baseline VD levels, which became even lower by the end of the study in the subjects who did not receive VD supplementation. The presence of VD deficiency or insufficiency is a common finding in pediatrics,[19] and seems to suggest a need for VD supplementation and nutritional support throughout childhood, especially during the winter months when its synthesis in skin exposed to the UV B radiation of sunlight is particularly reduced. However, the low VD levels played no role in conditioning the immune response to TIV because the immune response of the subjects with VD deficiency or insufficiency was similar to that of the children with normal baseline VD levels. Moreover, there was no difference between the children with persistently low levels and those whose levels normalized as a result of supplementation.

Our finding that VD levels had no effect on immune responses to TIV conflicts with the finding of Chadha et al. that baseline VD levels had a significant effect in adults with cancer when tested as a continuous variable in relation to serological response.[10]

Table 3. Immune responses to a trivalent influenza vaccine in a previously unvaccinated pediatric population by vitamin D levels after vitamin D treatment

Immune response	Vitamin D < 20 ng/mL (n = 36)	Vitamin D 20–29.9 ng/mL (n = 33)	Vitamin D ≥ 30 ng/mL (n = 47)	P value
A/H1N1v				
Seroconversion, No. (%)	16 (44.4)	18 (55.5)	17 (36.2)	0.26
Seroprotection (HI ≥ 40 at T1), No. (%)	27 (75.0)	28 (84.8)	38 (80.8)	0.58
Seroprotection (HI ≥ 110 at T1), No. (%)	18 (50.0)	19 (57.6)	24 (51.1)	0.79
Median GMT at T1 (range)	200 (10–2,560)	160 (5–1,280)	160 (5–2,560)	0.98
A/H3N2				
Seroconversion, No. (%)	21 (58.3)	18 (55.5)	23 (48.9)	0.69
Seroprotection (HI ≥ 40 at T1), No. (%)	30 (83.3)	30 (90.9)	37 (78.7)	0.35
Seroprotection (HI ≥ 110 at T1) No. (%)	24 (66.7)	22 (66.7)	25 (53.2)	0.34
Median GMT at T1 (range)	160 (10–2,560)	160 (5–5,120)	160 (5–2,560)	0.74
B				
Seroconversion, No. (%)	10 (27.8)	11 (33.3)	12 (25.5)	0.74
Seroprotection (HI ≥ 40 at T1), No. (%)	11 (30.6)	13 (39.4)	15 (31.9)	0.70
Seroprotection (HI ≥ 110 at T1), No. (%)	5 (13.9)	3 (9.1)	2 (4.3)	0.30
Median GMT at T1 (range)	10 (5–640)	10 (5–320)	10 (5–160)	0.63

P value between groups calculated using the x^2 or Kruskall-Wallis test as appropriate. GMT, geometric mean titer; HI, hemagglutination-inhibiting antibodies T1, end of the treatment period, three months after the last TIV administration.

However, the population enrolled in that study is quite different from ours and this may explain the different results. By definition, the characteristics of the immune system of cancer patients are different, and this may lead to a different response to VD administration. Moreover, the mean serum VD levels of the patients in the study of Chadha et al. were significantly higher than those of our children, even at the end of the treatment period. Furthermore, the positive impact of VD administration in the study by Chandra et al. was clear only in the case of the A/H3N2 antigen, whereas the responses to A/H1N1 and B strains were no different in patients whose VD levels were in the lowest and highest quartiles. Finally, given the small sample size, it is possible that the apparent effect of VD on the response to the A/H3N2 antigen was due to chance.

In conclusion, our findings indicate that the poor immunogenicity of TIV in younger children cannot be overcome by the simultaneous administration of VD. However, it cannot be excluded that higher VD doses administered significantly earlier than TIV may modify the immune response to the vaccine. Further studies of these variables are needed before any definite conclusions can be drawn.

Patients and Methods

This prospective, randomized, single-blinded, placebo-controlled study was performed in the outpatient pediatric clinic of the University of Milan's Department of Pathophysiology and Transplantation between October 1, 2011 and April 30, 2012. It was approved by the Ethics Committee of the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico in Milan, and was conducted in accordance with the standards of Good Clinical Practice for trials of medicinal products in humans; the children's parents or legal guardians gave their written informed consent before the children were enrolled.

The study involved children aged 2–5 y with a history of recurrent AOM (defined as at least three episodes in the preceding six months, or at least four episodes in the preceding 12 mo, with the most recent episode in the previous 2–8 weeks) who had not been previously vaccinated against influenza. At the time of enrolment, the children had to be free of any clinically evident febrile infectious disease. The other exclusion criteria were severe atopy, acquired or congenital immunodeficiency, the recent administration of blood products, the presence of anatomical abnormalities capable of favoring the development of AOM, and chronic treatment with drugs capable of interfering with the absorption or metabolism of VD, such as barbiturates, corticosteroids and cholestyramine.

The enrolled children were randomly divided into two groups and assigned to receive daily VD 1,000 IU (four drops of Dibase, Vitamin D_3, Abiogen Pharma S.p.A.) or placebo orally for four months. The study was single blinded because investigators knew whether the children were receiving VD or placebo, but parents were not aware.

The incidence of new episodes of AOM had to be monitored for six months after enrolment, and all of the children had to return to the center for a control visit every month or when a febrile episode occurred; furthermore, the parents had to complete a diary recording any clinical problems affecting the patients and the daily administration of VD. Compliance with the study regimen was verified by referring to these diaries and inspecting the returned medication bottles at the end of each month. Enrolment was completed at the end of October.

As part of the program designed to reduce the risk of new episodes of AOM, all of the children received a TIV (Fluarix,

GlaxoSmithKline Biologicals) by means of an injection in the deltoid muscle. The vaccine was formulated in accordance with the strain recommendations for the 2011–2012 northern hemisphere influenza season. Each dose consisted of 15 μg each of A/California/7/2009(H1N1)-like, A/Perth/16/2009(H3N2)-like and B/Brisbane/60/2008(B)-like purified influenza surface antigen neuraminidase and hemagglutinin with solvent added to reach 0.5 mL. All of the children received a dose of the vaccine at the same time as the first dose of VD or placebo; a second vaccine dose was administered 29 ± 2 d later. Before vaccine and VD/placebo administration, a blood sample was drawn in order to determine blood VD concentrations and the immunogenicity of TIV; a second blood sample was obtained at the end of the treatment period, three months after the last TIV administration.

Immunogenicity was evaluated by means of standard assay of hemagglutination-inhibiting (HI) antibodies to the influenza strains contained in the vaccine.[20] The serum samples were tested in duplicate at an initial dilution of 1:10, and those that were negative for the antibody were assigned an arbitrary titer of 1:5. HI antibody titers were expressed as the reciprocal of the highest serum dilution that completely inhibited hemagglutination. In accordance with the criteria described in the European Agency for the Evaluation of Medicinal Products (EMEA) guideline,[21] humoral immune response was assessed on the basis of the seroconversion rate (defined as the percentage of subjects experiencing at least a 4-fold increase in a seropositive pre-vaccination HI antibody titer, or an increase from < 10 to ≥ 40 in those who were seronegative), the seroprotection rate (defined as the percentage of subjects reaching an HI titer of ≥ 40) and geometric mean titers (GMTs). Moreover, as these criteria were developed for adults and it has recently been reported that a higher correlate of protection (an HI titer of ≥ 1:110) may be more appropriate for children,[22] seroprotection was also evaluated using this cut-off value.

VD was measured as 25-hydroxyVD by means of a chemiluminescence immunoassay (LIAISON 25 OH Vitamin D Total Assay, DiaSorin). Serum levels were considered normal if they were ≥ 30 ng/mL, insufficient if they were between 20 and 20.9 ng/mL and deficient if they were < 20 ng/mL.

In relation to influenza antigens, we hypothesized that seroconversion for children in the placebo group would have been equal to 65%. In consideration of this assumption, the study was designed to detect a 25% increase in seroconversion for children in the vitamin D group as compared with those in the placebo group, with 90% power and a two-sided type I error of 0.05, and keeping into account a 15% of subjects lost to follow-up. Therefore, about 55 children were enrolled in each group.

The continuous variables are expressed as median values and ranges, and the categorical variables as numbers and percentages. The continuous data were analyzed using a two-sided Wilcoxon rank-sum or Kruskall-Wallis test, as appropriate. The categorical data were analyzed using contingency table analysis and the chi-square or Fisher's exact test, as appropriate. All of the analyses were two-tailed, and p values of ≤ 0.05 were considered significant.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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